Developing and optimizing single tissue sample preparation for parallel global metabolomics and lipidomics

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INTRODUCTION

Limited amount of tissue sample often represents a big challenge for multiomics approaches. This is particularly difficult with a small amount of valuable samples given the invasive nature of sample collection. Another issue is that the tissue sample is not homogeneous throughout and the distribution of different types of cells (e.g., healthy and cancerous) varies. Therefore, dividing one sample into different parts for omics analyses can lead to different and/or misleading results. A key objective of this study focused on a sample preparation strategy to enable a simultaneous analysis of a single tissue sample by both metabolomics and lipidomics LC-MS analyses.

METHODS & RESULTS

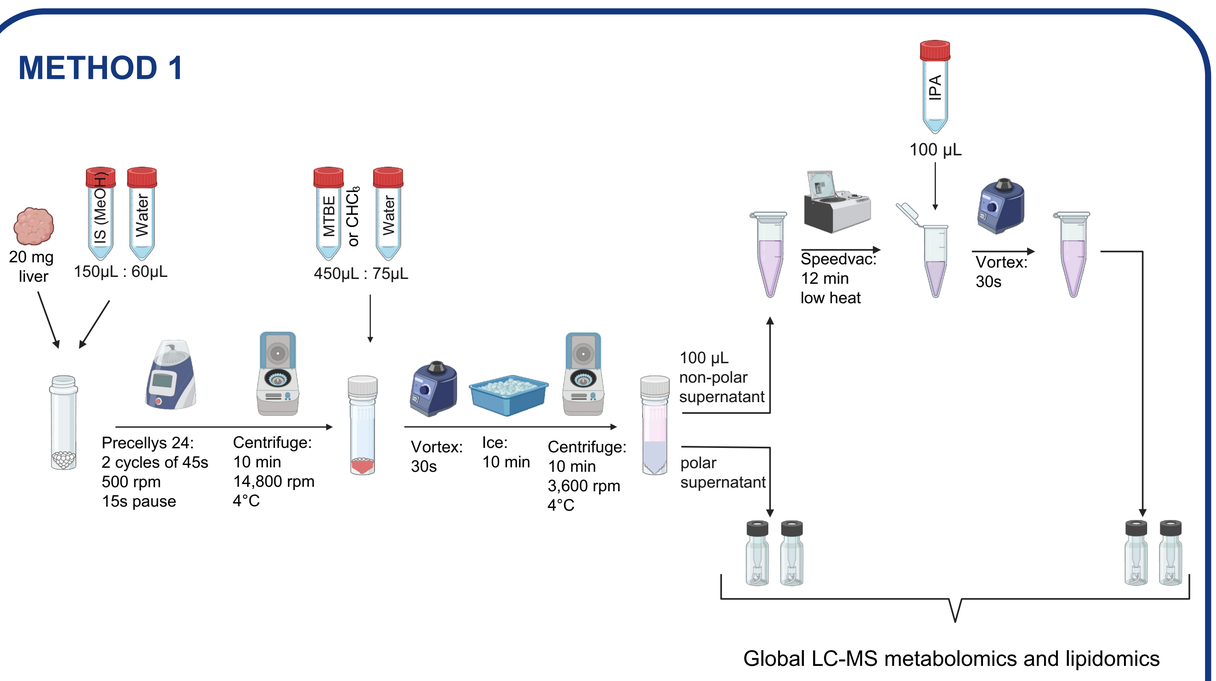
Sample preparation: A homogeneous liver tissue sample was selected to minimize biological variability across replicates. To monitor phase separation behavior, isotopically labeled internal standards (ILIS) representing both hydrophilic and hydrophobic species were included in all procedures. **Method 1** involved a two-phase extraction using either chloroform ($CHCl_3$) or methyl tert-butyl ether (MTBE), following established protocols. **Method 2** employed a single-phase extraction using methanol or isopropanol. In **Method 3**, tissue homogenization was initially performed in methanol. After centrifugation, the supernatant was collected, and the remaining pellet and residual supernatant were subjected to a secondary extraction with isopropanol.

Global LC-MS Metabolomic Analysis: Samples were analyzed using the Ultimate 3000 HPLC coupled to Q-Exactive Orbitrap MS. Separation of metabolites was done using XRs Diphenyl column (250 x 2 mm, 3 µm, Agilent) . All samples were analyzed in positive and negative ionization mode.

Global LC-MS Lipidomic Analysis: Lipidomic analysis was performed using Vanquish HPLC, Accucore C30 column (150 x 2.1 mm,2.6 µm) and Orbitrap Fusion Tribrid MS (all Thermo Scientific).

Data processing and statistical analysis: Compound Discoverer (3.3, Thermo Scientific) was used for MS data processing. Values of CV of ILIS were calculated for each sample preparation method.

METHOD 1



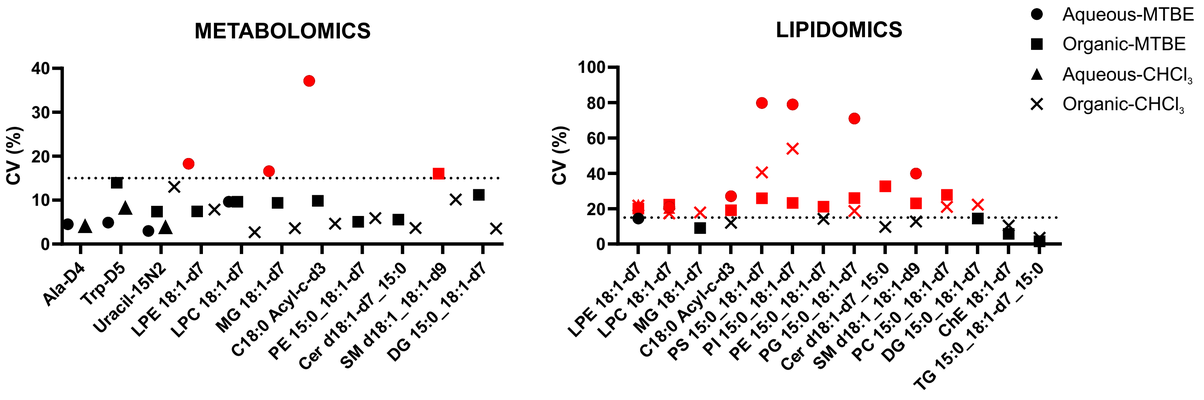
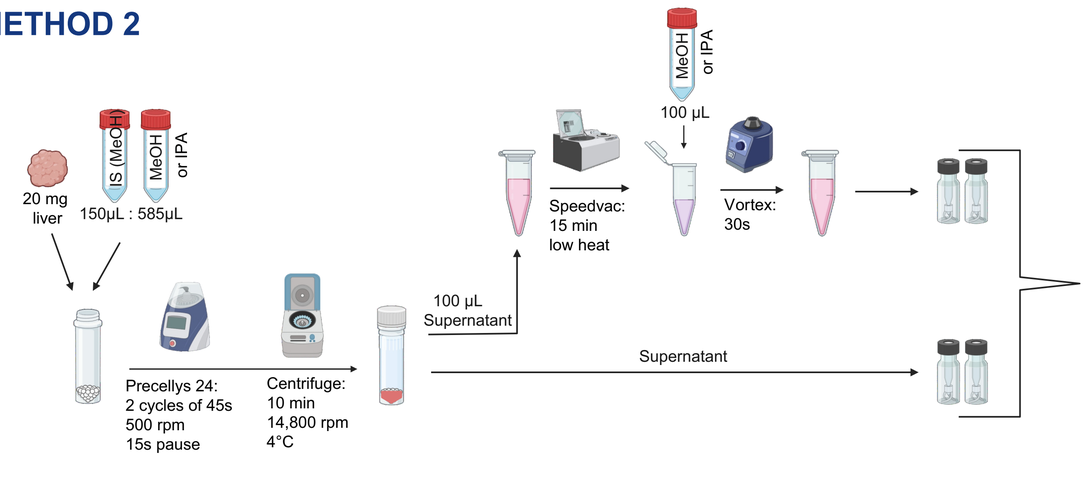


Figure 1. *Sample preparation workflow for the method 1. Liver tissue (n=5) was homogenized using water and ILIS in methanol and Precellys 24. After centrifugation, metabolites were extracted using either MTBE or $CHCl_3$. After centrifugation, fraction containing organic solvent was evaporated and dissolved in isopropanol. Both fractions were analyzed using global LC-MS metabolomics and lipidomics. To evaluate precision of this extraction approach, coefficients of variation (CV) for ILIS were calculated. Fractions with CV >15 % are highlighted by red colour.*

METHOD 2



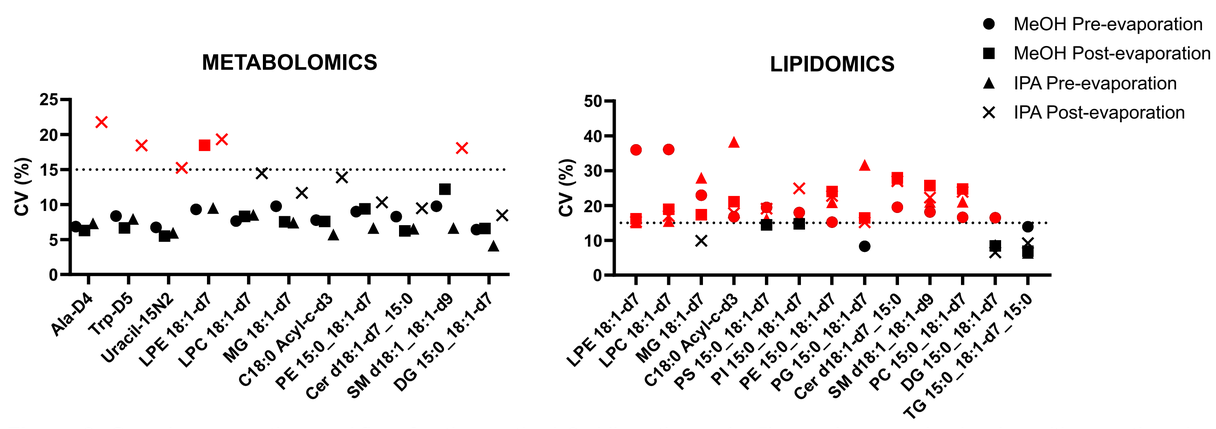
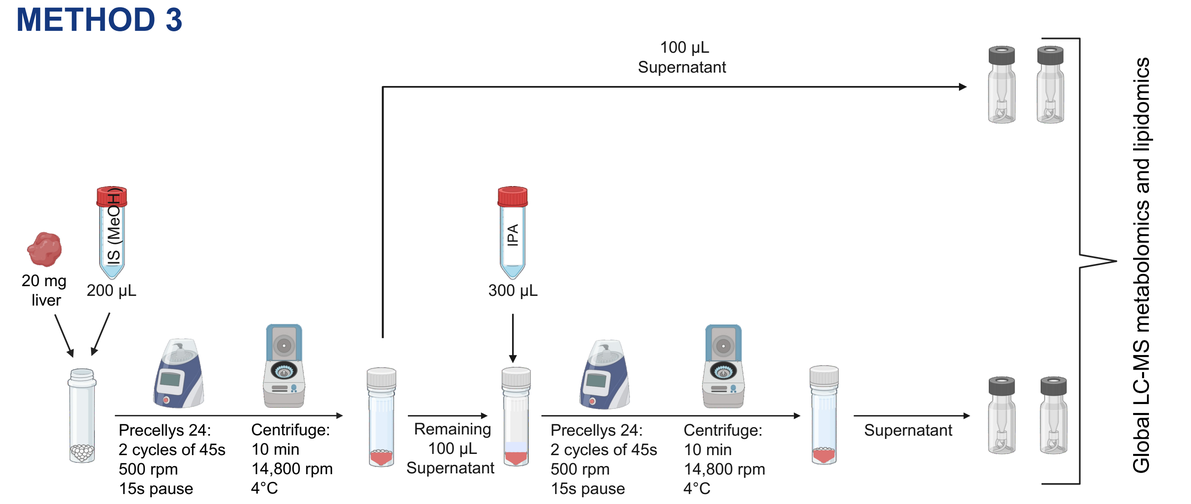


Figure 2. *Sample preparation workflow for the method 2. Lliver tissue (n=5) was homogenized using either methanol or isopropanol, ILIS in methanol and Precellys 24. After centrifugation, one fraction was transferred to HPLC glass vial. Another fraction was evaporated and metabolites were dissolved either in methanol or isopropanol, the same solution used in the first step. Both fractions were analyzed using global LC-MS metabolomics and lipidomics. To evaluate precision of this extraction approach, coefficients of variation (CV) for ILIS were calculated. Fractions with CV >15 % are highlighted by red colour.*

METHOD 3



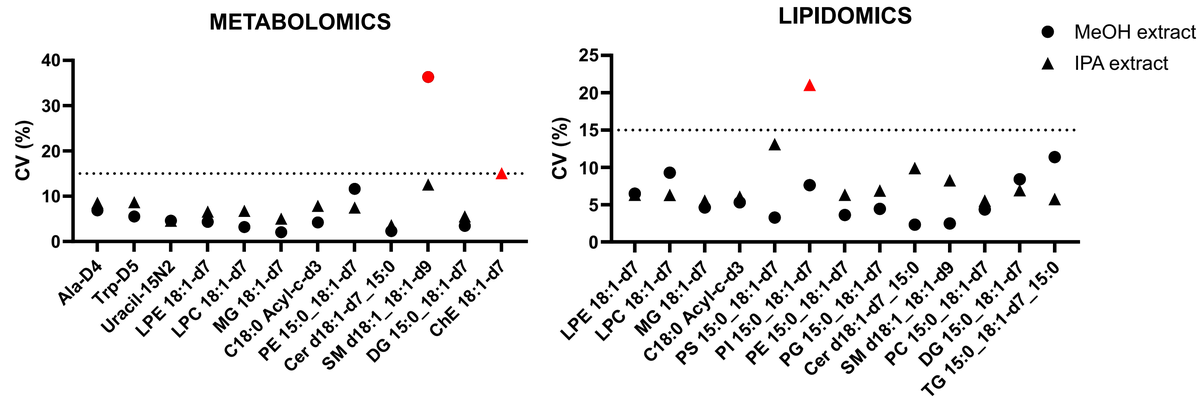


Figure 3. *Sample preparation workflow for the method 3. Lliver tissue (n=5) was homogenized using ILIS in methanol and Precellys 24. After centrifugation, part of this methanolic fraction was transferred to HPLC glass vial. To the rest of the methanolic fraction with precipitated proteins, isopropanol was added and sample was homogenized again. After centrifugation, this supernatant was also transferred to HPLC glass vial. Both fractions were analyzed using global LC-MS metabolomics and lipidomics. To evaluate precision of this extraction approach, coefficients of variation (CV) for ILIS were calculated. Fractions with CV >15 % are highlighted by red colour. Linearity of this extraction approach was also tested (Figure 4C).*

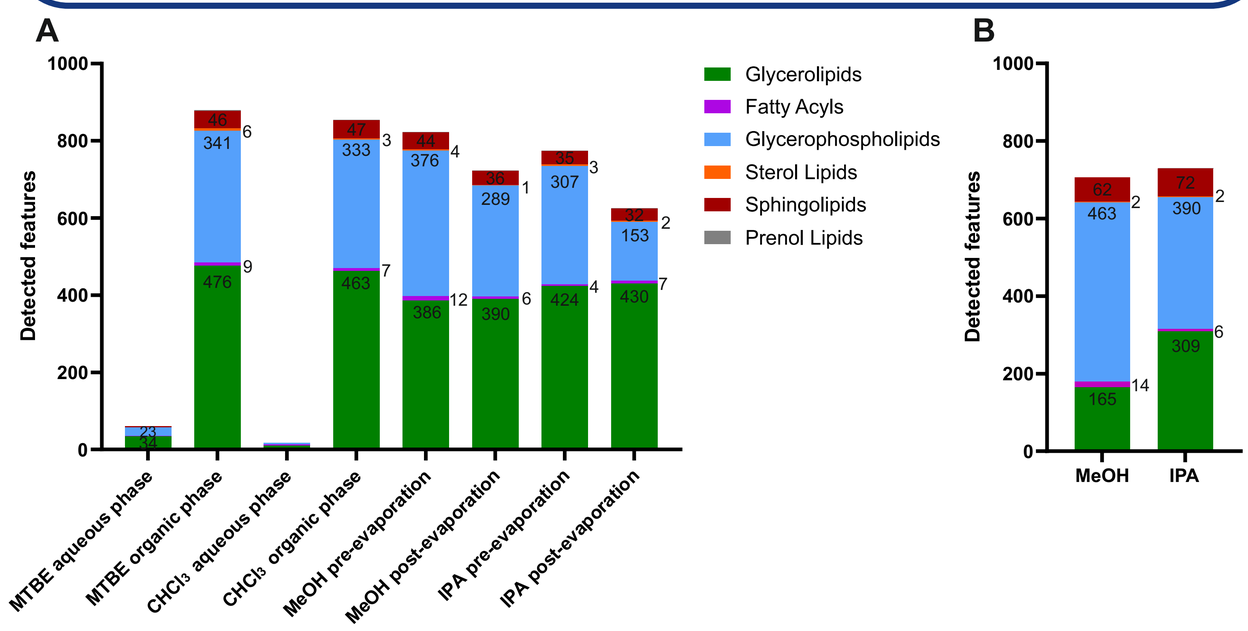


Figure 4. *Detected lipid features using method 1 and 2 (A) and method 3 (B)The detected lipid species were classified into the main lipid classes according to LIPID MAPS. For the sample preparation method 3 (C) linearity was assessed by extracting increasing amounts of liver tissue (10–80 mg) using a constant extraction volume (200 µL) in five replicates. Normalization level (NL) of TIC was then recorded.*

CONCLUSION

Several tissue sample preparation approchaes were tested and ILIS were included to evaluate the precision of all methods. Regarding the CV values, method 3 showed the best results where only 2 (metabolomics) and 1 (lipidomics) ILIS resulted in CV above 15 %. The high variation of ILIS throughout the methods can be explained by their various solubility in different solutions used. Also, the integration of evaporation steps had a negative impact on precision of the sample preparation methods and number of lipid species. Method 3 also showed a good linearity demonstrated by increasing the liver tissue amount while keeping a constant extraction volume. For lipidomics, the normalization level of the TIC increased proportionally across the full range. In contrast, metabolomics showed a near-logarithmic response with plateauing of the TIC at tissue amounts above 40–60 mg, likely due to ion suppression and matrix effects. The results suggest that tissue amounts up to 60 mg in 200 µL extraction volume provide optimal balance for both lipidomic and metabolomic analyses.